



NTP
National Toxicology Program

NTP High Throughput Screening Assays Workshop

December 14 - 15, 2005

Chair: Dr. Shuk-Mei Ho, University of Cincinnati





Breakout Groups

- **Selection of Targets and Assays for High Throughput Screening (HTS)**
 - **Co-chairs:** Dr. Kate Johnston (Cellumen Inc.), Dr. Tim Zacharewski (Michigan State Univ.)
 - **Members:** Dr. Kim Boekelheide (Brown Univ.), Dr. Christopher Bradfield, Univ. Wisconsin), Dr. Richard Brennan (Iconix Pharmaceuticals), Dr. Jim Inglese (NIH), Dr. Jeffrey Lawrence (Merck and Co., Inc.), Dr. Charlene McQueen, Univ. Arizona), Ms. Kristine Witt (NTP)
- **Conduct of Studies including Chemical Selection, Study Design and Analytical Methods**
 - **Co-chairs:** Dr. Christopher Lipinski (Pfizer Global R&D), Dr. William Janzen (Amphora Discovery Corp.)
 - **Members:** Dr. Gabriela Chiosis (Memorial Sloan-Kettering Cancer Center), Dr. Tina Garyantes (Sanofi-Aventis), Dr. Ken Lewis (OpAns, LLC), Dr. Douglas Livingston (Discovery Partners Inc.), Dr. Kevin Oldenburg (Matrical Inc.), Dr. Cynthia Smith (NTP), Dr. Raymond Tice (NTP)
- **Data storage, Analysis and Interpretation**
 - **Co-Chairs:** Dr. Pauline Gee (CeMines Inc.), Dr. Alexander Tropsha (Univ. North Carolina at Chapel Hill)
 - **Members:** Dr. Kenneth Crump (Environ Inc.), Dr. Nigel Greene (Pfizer Global R&D), Mr. Ajit Jadhav (NIH), Dr. Ann Richard (EPA), Dr. Keith Soper (Merck Research Laboratories)
- **Application of Data from HTS Assays in Regulatory Decision-making**
 - **Co-Chairs:** Dr. Jonathan Freedman (NIEHS), Dr. Hillary Carpenter (Minnesota Department of Health)
 - **Members:** Dr. William Allaben (NCTR, FDA), Dr. Richard Becker (American Chemical Council), Dr. David Dix (EPA), Dr. Jennifer Sass (National Resources Defense Council), Dr. Philip Sayre (EPA), Ms. Kristie Stoick (Physicians Committee for Responsible Medicine), Dr. William Stokes (NTP), Dr. Mark Toraason (NIOSH), Dr. Stanley Young (National Institute for Statistical Sciences)



Group 1: Identify targets and assays for HTS - Conceptual Approach

Guiding principles

- **Utilize transcript profiling to guide selection of pathways and targets**
- **Require multi-dose, multi-time point assays**
- **Use most physiologically-relevant cells with metabolizing capabilities e.g., MCL5 or co-cultures**
- **Use primary cultures of both rodent and human cells or non-transformed cell lines**



Most important pathways for carcinogenesis

- **Apoptosis, both pro- and anti-**
- **Proliferation, cell-cycle control**
- **DNA damage and repair**
- **Chromatin remodeling**
- **Signal transduction modulation**



Group 2: Study design, chemical selection, analytical methods

Study design - ideal

- **Dose response curve required; replicates on each plate**
- **Test on multiple days**
- **Replicate in different labs**
- **Use cells with cloned p450/other enzymes, or hepatocytes, to mimic mammalian metabolism**
- **Run standards daily in each experiment, randomly arrayed on plate**
- **Concurrent controls:**
 - **Required on every plate**
 - **Representative of strong and weak response**
 - **Use historical control values for QC**



Chemical selection (priority, stability and handling)

- **Compounds with known toxicity profiles and unknowns of high public health priority**
- **Include metabolites if known and available**
- **Use highest concentration of chemical that is soluble and not cytotoxic (in cell-based assays)**
- **Stability and solubility of chemicals can be problematic, but**
- **Do not verify concentration of chemical in well**
- **Group chemicals by characteristics to assist in handling**
- **Need for chemoinformatics methods and database**



Analytical methods (general limitations)

- **Cannot test volatiles**
- **Solvents can be toxic**
- **Must minimize false negatives**
- **General methods used for drug-like chemicals may not work for compounds of interest to NTP**



Group 3: Data storage, analysis and interpretation

Data storage

- **The HTS data for the NTP sets of compounds will be stored in same format as the NIH data in PubChem, a publicly accessible database**
- **The data should be stored in their:**
 - **Original and raw form**
 - **Normalized and summarized**
 - **With appropriate annotation including skeleton protocol**
- **Database must be easy to access and data easily retrieved**



Analysis of HTS Data

- **Normalize the data according to the specific protocol of the assay and within each plate if internal controls are used**
- **Statistical analysis must incorporate plate-to-plate variation**
- **Linkage of HTS data with NTP bioassay data must occur to begin to build an engine to predict outcomes sufficiently well to use in priority setting**
- **This requires:**
 - **Translation of CAS numbers to standard machine readable structures so bioassay data can be retrieved at the same level as the HTS data**
 - ***Organization of In vivo* data in a uniform ontology framework of pathology (and other domains) consistent across all studies in machine readable language**



Group 4: Application of data from HTS assays in regulatory decision-making

Presently

- **Data from HTS assays cannot be used for making regulatory decisions**
- **Could be used in priority setting**
- **What is required?**
 - **Validation**
 - **Uncertainty analysis**
 - **Predictive ADME**
 - **Test in large number of assays**

Outreach Program

- **Workshops and training needed for people in the regulatory agencies that will review and evaluate HTS data**



Acceptance of HTS Assays for Regulatory Decisions

What are the criteria for regulatory acceptance?

- **Relevant (fits into exposure-disease continuum)**
- **Reliable (a workable assay)**
- **Repeatable (consistent results within/among labs)**
- **Recognized (acceptance by a large diverse audience)**
- **Realistic (outcome used for decision-making).**
- **Predictability - must provide information on sensitivity and specificity for a well-characterized reference database of test agents**
- **In 3-5 years, there is potential for chemical grouping by assay results, and prioritization**